The Journal of Animal & Plant Sciences, 28(2): 2018, Page: 441-448
ISSN: 1018-7081

EFFECT OF NATURAL ASCORBIC ACID ON PERFORMANCE AND CERTAIN HAEMATO-BIOCHEMICAL VALUES IN LAYERSEXPOSED TO HEAT STRESS

Alaeldein M. Abudabos[1*], Abdullah N. Al-Owaimer[1], Elsayed O.S. Hussein[1], Mutahar H. Ali[1], and Muath Q. Al-Ghadi[2]

[1]Department of Animal Production, College of Food and Agriculture Sciences; [2]Department of Zoology, College of Science,King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia.
Corresponding author's email: aabudabos@ksu.edu.sa

ABSTRACT

A four week trial examined the effects of dietary vitamin C supplement (200 mg/kg) and heat stress on performance of layers and blood biochemical profile. Two levels of environmental temperatures: thermoneutral (TN, 22°C) and high temperature (HT, 32°C) and two levels of vitamin C supplement (without and with) were arranged in a factorial arrangement resulted in four dietary treatments on feed intake (FI), egg mass (EM), feed conversion ratio (FCR), egg shell strength (ESS) and blood hematology in one strain of layers (Hy-Line W-36) from 65 to 69 weeks of age. Four treatments were assigned to nine replicates with three hens per pen. Treatment 1 was a corn-soy control in TN, treatment 2 was control in HS, treatment 3 was control + 0.2 g/l ascorbic acid in TN and treatment 4 was control + 0.2 g/l ascorbic acid in HS. Hens which were subjected to TN environment had higher FI as compared to those which had subjected to HS environment ($P < 0.05$). Egg mass was not affected by vitamin C, temperature or their interaction ($P > 0.05$). Feed conversion ratio was affected by temperature but not with Vitamin C supplementation. Hens which were subjected to TN had better FCR as compared to those which were subjected to HS for weeks two, three, four and cumulative period ($P\leq0.01$; $P\leq0.05$; $P\leq0.01$; $P\leq0.01$, respectively). Hens which were subjected to HS had weaker shell strength at week two ($P\leq0.01$), week four ($P\leq0.05$) and cumulative period ($P\leq0.001$). On the other hand, there was a significant interaction for vitamin C x Temperature at week three ($P\leq0.001$). Vitamin C increased Na and P concentrations in serum ($P\leq0.05$, $P\leq0.01$, respectively) and decreased Ca and P concentrations as compared to those which were subjected to TN ($P\leq0.05$, $P\leq0.05$, respectively). It can be concluded that natural vitamin C did not have a major impact on cumulative performance or plasma mineral status.

Key words: Heat stress, thermoneutral, vitamin C, layers, Feed conversion ratio (FCR), Hen performance,

INTRODUCTION

Heat stress has a negative impact on economic losses in poultry production both for tropical and temperate areas (Al-Saffar and Rose, 2002; Khan *et al.*, 2011). Heat stress remains a continuous challenge for the layer enterprises during summer season when the area is exposed to recurrent summer heat waves (Khan *et al.*, 2012; Chand *et al.*, 2016). Layers are predominantly susceptible to heat stress because they have to maintain a long production cycle which could extend from 50 to 70 weeks (Mignon-Grasteau *et al.*, 2015).

High environmental temperature, high humidity and low air velocity are factors contribute to heat stress (Balnave and Brake, 2005; Khan *et al.*, 2014a). Heat stress arises when layers struggle in balancing body heat production and body heat loss. Layers depend on several mechanisms to regulate their body temperature within the thermo neutral zone when they are subjected to high environmental temperatures (Simon, 2003). To avoid heat stress, the thermoregulatory mechanisms in poultry are normally stimulated above the thermoneutral zone of 24◦C (Celik *et al.*, 2004; Chand *et al.*, 2017). Heat stress effects become noticeable when temperatures exceed 30◦C (Seven, 2008).

Heat imposes severe stress on layers, which leads to poor performance, depresses body weight, egg production, egg weight, shell quality and is generally accompanied by suppression of feed intake (Mashaly *et al*, 2004; Chand *et al.*, 2014),predisposing birds to various infectious diseases and high mortality rates (Tanor *et al.*, 1984, Maini *et al.*, 2007). Corticosteroid secretion increased in response to stress, corticosteroid has a catabolic effect and cause impairment of cellular functions (Ramnath *et al.*, 2008; Khan *et al.*, 2013). Also, most of the production energy is diverted to thermoregulatory adaptations during the periods of heat stress which results in oxidative stress induced immune-suppression (Maini *et al.*, 2007).

There are various strategies to minimize the negative effects of heat stress on layer hens by modification of their diets (Ajakaiye *et al.*, 2010; Raza *et al.*, 2016).Numerous studies have documented the beneficial effects of vitamin supplementation on egg production and eggshell quality in stressed poultry C (Waseem *et al.*, 2008, Torki *et al.*, 2014). In birds, it was postulated that ascorbic acid stimulates 1,25-dihydroxy-cholecalciferol and together with it increases calcium mobilization from bones, suggesting that vitamin C has

an important role in eggshell formation (Dorr and Balloun, 1976).

The body requirement of ascorbic acid during heat stress in poultry is greater than the amount synthesized by normal tissues and its administration to broilers during heat stress has been shown to be beneficial to the body (Balogun *et al.*,1996; Khan *et al.*, 2012).Poultry are renal synthesizers of ascorbic acid (AsA) (Maurice *et al.*, 2002) and diets are not normally fortified; hence, no recommended requirement is established by the NRC (NRC, 1994). It is generally assumed that the endogenous synthesis is sufficient to meet biological demands in poultry; however, environmental stressors are known to alter AsA use or synthesis in the poultry (Pardue and Thaxton, 1986; Khan *et al.*, 2014b).

The objective of the current study was to evaluate the efficacy of natural vitamin C on performance and blood hematoogy of laying hens exposed to cyclic heat stress for the period from 65 to 69 weeks of age.

MATERIALS AND METHODS

Experimental Design and Diets: Two levels of temperature [thermoneutral (TN, 22°C) and high temperature (HT, 32°C)] and two levels of vitamin C supplement (without and with) were applied in a factorial arrangement for a total of four dietary treatments. Birds were kept in 36 pens in two different rooms, with 18 pens per room. One room was assigned a treatment classified as thermoneutral (TN) while the second room was designated as high temperature (HT). High temperature cycle was applied in the HT room. On daily basis, the temperature in the HT room was allowed to cycle between 22°C and 32°C, while temperature in the TN room was maintained at 22°C, throughout the rest of the study. The HT birds were exposed to 32°C from 8:00 to 15:00 then temperature was steadily decreased to 22°C.

All fourtreatments were fed to one strain of hens (Hy-line) from 65-69 weeks of age for a total time period of four weeks. Each treatment was assigned to 9 replicate pens with 3 hens per pen (412 cm^2 / hen) for a total of 36 pens. The multi-bird pen was the experimental unit. The study was conducted under a protocol approved by King Saud University and complies with the current laws of Saudi Arabia.

Diets were formulated to meetphase III of the Hy-line strain recommendations (Table 1). Treatment 1 was the control diet (standard corn-soybean meal) and birds were kept in TN environment. Treatment 2 was the control diet and birds were kept in HS environment. Treatments 3 and 4 were supplemented with 0.2 g /l AsA and birds were kept in TN and HS environments, respectively. The supplement used in this experiment was a commercial vitamin C supplement derived from amla (VC-100).

Measurements

Hen,Egg and Blood parameters: Feed consumption and egg production were measured daily, and egg production calculated on henday basis. Egg mass was calculated as a factor of egg weight and egg production. All hens were individually weighed every 4 weeks. Feed conversion was calculated as g of feed: g of egg mass.

Blood samples were withdrawn from five randomly selected birds per treatment via brachial venipuncture into EDTA tubes for hematological analysis, and into plain tubes for serological analysis. EDTA tubes were used to analyze various blood parameters such as white blood cell counts (WBC), total red blood cell counts (RBC), hemoglobin content (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), standard deviation in red cell distribution width (RDW-SD), coefficient variation of red cell distribution width (RDW-CV), hematocrit (HCT), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT). Hematological parameters were determined by methods described by Campbell (1988). Serum was prepared by centrifuging plain tubes at 5 ºC and 3000 rpm for 10 min. Thereafter, sera were transferred into eppendorf tubes and stored at -20ºC until further analysis. Serum levels of sodium (ion-selective method), chlorine (Labtest method), magnesium (Tonks' method), total calcium (Labtest method) and phosphorus (Basques-Lustosa's method) were determined using commercial kits (M di Europa GmbH Wittekamp 30. D-30163 Hannover, Germany).

Statistical Analysis: Analysis of variance was performed using GLM procedure of SAS (SAS, 2009) for randomized complete block design with 2 x 2 factorial arrangements of treatments, in which each treatment was assigned to 9 replicate pens. The data were tested for main effects of vitamin C (AsA), temperature (temp.) and for interaction effect for AsA x Temp. Statistical significance was assessed at ($P \leq 0.05$).

RESULTS AND DISCUSSION

Vitamin C supplementation had no significant effect on feed intake during the experiment period (Table 2). However, the results are not consistent with those of Torki *et al.* (2014) who reported that feed intake improved in layers when they were given 250 mg/kg vitamin C. Temperature had an effect on feed intake for weeks one, two, four as well as at the cumulative period of the experiment ($P \leq 0.05$).Hens which were subjected to TN environment had higher feed intake as compared to those subjected to HS (Table 2). Vitamin Cand

temperature interaction showed no effect on FI (P> 0.05). In the present study, heat stress reduced feed intake. These findings confirm earlier studies (Star *et al.*, 2008). It was estimated that feed intake decreased by 1.6% for each one degree temperature rise between 17 and 29°C and the decreased feed intake was explained by a drop in energy requirement rather than an effect of increased temperature *per se.* Daniel and Balnave (1981) reported that heat stress had more effect on feed intake of laying hens as compared to egg production and concluded that hens utilized the feed more efficiently under heat stress.

Egg mass was not affected by vitamin C, temperature or their interaction (P>0.05) (Table 3).These results are in general agreement with the results of Puthpongsiriporn *et al.* (2001) who reported similar egg production and egg mass of laying hens which receiving two concentrations of vitamin C (0 to 65 mg/kg diet). The present results disagree with those of Keshavarz (1996), who reported that egg mass increased as a result of supplementing the diets of heat-stressed laying hens with 250 mg/kg vitamin C.

Feed conversion ratio (FCR) was affected by temperature (Table 4). Hens subjected to TN had better FCR as compared to those which were subjected to HS for weeks two, three, four and cumulative period (P≤0.01; P≤0.05; P≤0.01; P≤0.01, respectively). Vitamin C supplementation had no effect on FCR. Similarly, Torki *et al.* (2014) reported that 250 mg/kg vitamin C had no impact on FCR in layers.

Egg shell strength was significantly affected by temperature for weeks two, four and the cumulative period (Table 5). Hens which were subjected to HS had weaker shell strength at week two (P≤0.01), week four (P≤0.05) and cumulative period (P≤0.001). The effect of heat stress on egg shell quality has been well documented (Miller and Sunde, 1975; Wolfenson *et al.,* 1979). de Andrade *et al.*(1976) found that shell quality was not maintained at constant elevated temperatures. A fall in plasma calcium level was speculated to be the cause for the lower shell quality. In the present study, egg shell quality was not maintained during the period of heat stress nor during the two following periods (7 days).It was significantly (P≤0.05) affected by both diet and temperature.

Table 6 shows the effect of treatment on mineral concentration. Vitamin C affected Na and P concentrations (P≤0.05, P≤0.01, respectively). Vitamin C increased Na concentration from 4.3 to 16.1 mmol/l and increased P concentration from 9.1 to 11.4 mg/dl. On the other hand, temperature affected Ca and P concentrations (P≤0.05, P≤0.05, respectively). Hens which were subjected to HS had lower Ca and P concentrations as compared to those which were subjected to TN (14.0, 19.0 mg/dl for Ca and 9.8, 11.4 mg/dl for P, respectively).Effect of treatment on blood hematology is presented in Table 7. Vitamin C affected only MCH and MCHC (P≤0.001, P≤0.01).

Table 1. Composition of the basal diets

Ingredient	**(%)**
Corn	65.8
Soybean meal	15.9
Corn gluten meal	5.9
Corn oil	1.0
Limestone	7.4
Dicalcium phosphate	1.7
Oyster shell	1.5
Salt	0.5
DL-Methionine	0.05
Lysine	0.16
Trace mineral[1]	0.16
Vitamin premix[1]	0.05
Nutrient analysis	
Protein calculated, %	16.5
Protein analyzed, %	16.7
M.E., Kcal/Kg	2860
Calcium calculated, %	4.0
Calcium analyzed, %	3.9
Avail. Phosphorus, %	0.40
TSAA, %	0.66
Lysine, %	0.85

[1]Mineral- vitamin premix provided Mn, 88; Cu, 6.6 mg; Zn, 88 mg; Se, 0.3 mg; vitamin A, 6,600 IU; cholcalciferol, 2,805 IU; vitamin E, 10 IU; vitamin k, 2.0 mg; Riboflavin, 4.4 mg; pantothenic acid, 6.6 mg; niacin 24.2 mg; choline, 110 mg; vitamin B12, 8.8 mg; ethoxyquin, 1.1 mg; per kg diet.

Table 2. Effect of dietary treatments on feed intake of layers exposed to heat stress

Diet	**Vitamin C**	**Temperature**	**W1**	**W2**	**W3**	**W4**	**Cumulative**
	(g/l)	(°C)			(g/d)		
1	0	22	114.1	112.1	110.0	110.9	111.8
2	0	32	107.8	101.2	103.0	102.8	103.7
3	0.2	22	118.0	112.3	110.9	114.2	113.9
4	0.2	32	109.4	106.1	106.2	103.5	106.3
SEM		0.1	3.2	3.8	3.1	3.7	2.9
Vitamin C average							
Without			110.9	106.6	106.5	106.9	107.7
With			113.7	109.2	108.6	108.8	110.1

SEM	2.2	2.7	2.2	2.6	2.1
Temperature average					
22 °C	116.1[a]	112.2[a]	110.4	112.6[a]	112.8[a]
32 °C	108.6[b]	103.6[b]	104.6	103.1[b]	105.0[b]
SEM	2.2	2.7	2.2	2.6	2.1
Statistical Probabilities					
Vitamin C	NS	NS	NS	NS	NS
Temperature	0.02	0.03	NS	0.018	0.013
Vitamin C x Temperature	NS	NS	NS	NS	NS

g/d: gram of feed per day,NS: not significant,SEM: standard error of the mean,W: week

Table 3. Effect of dietary treatments on egg mass of layers exposed to heat stress

Diet	Vitamin C	Temperature	W1	W2	W3	W4	Cumulative
	(g/l)	(°C)			(g)		
1	0	22	58.3	60.4	58.4	57.9	58.0
2	0	32	59.7	58.7	58.4	54.5	57.0
3	0.2	22	60.8	60.7	59.7	56.2	59.0
4	0.2	32	59.7	59.0	58.6	57.2	58.6
SEM			1.6	1.6	1.1	1.6	1.1
Vitamin C average							
Without			59.0	59.6	58.4	56.2	58.3
With			60.3	59.8	59.1	56.7	59.0
SEM			1.1	1.1	0.8	1.1	0.8
Temperature average							
22 °C			59.5	60.5	59.0	57.0	59.0
32 °C			59.7	58.9	59.5	55.9	58.2
SEM			1.1	1.1	0.8	1.1	0.8
Statistical Probabilities							
Vitamin C			NS	NS	NS	NS	NS
Temperature			NS	NS	NS	NS	NS
Vitamin C x Temperature			NS	NS	NS	NS	NS

NS: not significant,SEM: standard error of the mean,W: week

Table 4. Effect of dietary treatments on feed conversion ratio of layers exposed to heat stress

Diet	Vitamin C	Temperature	W1	W2	W3	W4	Cumulative
	(g/l)	(°C)			(g: g)		
1	0	22	1.889	1.769	1.822	1.801	1.811
2	0	32	1.977	1.918	1.898	2.099	1.998
3	0.2	22	1.776	1.669	1.728	1.835	1.782
4	0.2	32	1.922	1.905	1.877	1.938	1.907
SEM			0.062	0.068	0.054	0.069	0.052
Vitamin C average							
without			1.933	1.843	1.860	1.950	1.905
With			1.849	1.787	1.803	1.887	1.845
SEM			1.1	1.1	0.8	1.1	0.8
Temperature average							
22 °C			1.832	1.719[b]	1.776[b]	1.818[b]	1.797[b]
32 °C			1.950	1.911[a]	1.888[a]	2.019[a]	1.953[a]
SEM			0.044	0.048	0.038	0.049	0.037
Statistical Probabilities							
Vitamin C			NS	NS	NS	NS	NS
Temperature			NS	0.008	0.046	0.007	0.005
Vitamin C x Temperature			NS	NS	NS	NS	NS

g/g: gram of feed per to gram of egg mass,NS: not significant,SEM: standard error of the mean,W: week

Table 5. Effect of dietary treatments on egg shell strength of layers exposed to heat stress

Diet	Vitamin C	Temperature	W1	W2	W3	W4	Cumulative
	(g/l)	(°C)			(mm)		
1	0	22	4.28	4.19	4.65	4.44	4.39
2	0	32	3.92	3.49	3.71	4.13	3.81
3	0.2	22	4.42	4.31	4.21	4.72	4.42
4	0.2	32	4.24	3.98	4.26	4.20	4.17
SEM			0.30	0.15	0.17	0.17	0.10
Vitamin C average							
without			4.10	3.84	4.18	4.28	4.10
with			4.33	4.15	4.24	4.46	4.29
SEM			0.21	0.11	0.12	0.12	0.07
Temperature average							
22 °C			4.35	4.25	4.43	4.58	4.40
32 °C			4.08	3.75	3.99	4.16	3.99
SEM			0.21	0.11	0.12	0.12	0.07
Statistical Probabilities							
Vitamin C			NS	NS	NS	NS	NS
Temperature			NS	0.002	NS	0.02	0.0004
Vitamin C x Temperature			NS	NS	0.0009	NS	NS

NS: not significant, SEM: standard error of the mean,W: week

Table 6. Effect of dietary treatments on blood mineral content of layers exposed to heat stress.

	Treatment				SEM	Vit C		SEM	Temperature		SEM	Probability		
	T1	T2	T3	T4		0	0.2		22°C	32°C		Vit. C	Tem.	Vit C x Tem.
Mineral concentration														
Na (mmol/l)	6.6	2.0	13.6	18.5	4.6	4.3	16.1	3.29	10.1	10.2	3.2	0.03	NS	NS
Cl (mmol/l)	99.3	99.0	96.3	94.5	3.2	99.1	95.4	2.29	97.8	96.7	2.3	NS	NS	NS
Mg (mmol/l)	3.4	3.2	3.4	2.9	0.17	3.3	3.1	0.12	3.43	3.08	0.12	NS	NS	NS
Ca (mg/ dl)	18.2	12.7	19.7	15.4	1.9	15.4	17.5	1.37	18.9	14.0	1.37	NS	0.034	NS
P (mg/ dl)	9.7	8.4	13.0	9.8	0.69	9.1	11.4	0.19	11.4	9.1	0.49	0.01	0.01	NS

Na: sodium, Cl: chloride, Mg: magnesium, Ca: calcium and P: phosphorus.NS: not significant, SEM: standard error of the mean, Tem: temperature.

Table 7. Effect of dietary treatments on blood hematology of layers exposed to heat stress.

	Treatment				SEM	Vit C		SEM	Temperature		SEM	Probability		
	T1	T2	T3	T4		0	0.2		22°C	32°C		Vit. C	Tem.	Vit C x Tem.
Hematology														
WBC ($\times10^3/\mu L$)	116.1	115.5	116.7	117.6	3.3	115.8	117.1	2.3	116.4	116.5	2.3	NS	NS	NS
RBC ($\times10^6//\mu L$)	4.0	3.9	3.5	3.8	0.18	3.9	3.6	0.1	3.7	3.8	0.13	NS	NS	NS
HGB (%)	35.9	34.7	33.4	36.8	1.5	35.3	35.1	1.0	34.6	35.7	1.0	NS	NS	NS
MCV (fL)	111.6	111.6	112.9	113.3	1.9	111.6	113.1	1.3	112.2	112.4	1.38	NS	NS	NS
MCH (pg)	88.9	88.1	94.3	96.2	1.1	88.5	95.2	0.84	91.6	92.1	0.84	0.0005	NS	NS
MCHC (gHb/100 ml)	79.6	79.0	83.5	84.4	1.0	79.3	83.9	0.73	81.6	81.7	0.73	0.002	NS	NS
RDW-CV (%)	9.3	9.6	9.5	9.7	0.2	9.5	9.6	0.17	9.4	9.6	0.17	NS	NS	NS
RDW-CD (fL)	41.6	43.0	43.3	44	1.3	42.3	43.6	0.97	42.5	43.5	0.97	NS	NS	NS
HCT (%)	45.1	44.0	39.9	43.4	2.3	44.5	41.7	1.6	42.5	43.7	1.65	NS	NS	NS
PLT ($\times10^3/\mu L$)	87.6	101.6	111.6	114.0	22.6	94.6	112.8	16.0	99.6	107.8	16	NS	NS	NS
MPV (fL)	10.8	11.1	11.3	10.3	0.2	10.9	10.8	0.14	11	10.7	0.14	NS	NS	0.01
PDW (%)	13.8	13.2	14.3	12.5	0.8	13.5	13.4	0.58	14	12.8	0.58	NS	NS	NS
PCT(%)	0.09	0.11	0.12	0.12	0.02	0.10	0.12	0.02	0.11	0.11	0.02	NS	NS	NS

WBC: white blood cell counts, RBC: total red blood cell counts, HGB: hemoglobin content, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW-SD: standard deviation in red cell distribution width, RDW-CV: coefficient variation of red cell distribution width, HCT: hematocrit, PLT: platelet count, MPV: mean platelet volume, PDW: platelet distribution width, PCT: plateletcrit, NS: not significant; SEM: standard error of the mean, Tem: temperature

Conclusion: High environmental temperature reduced feed intake and increased FCR for the cumulative period of the experiment. In the contrary, vitamin C supplementation had no influence on feed intake or FCR.Egg shell strength was deteriorated with high temperature. Vitamin C increased Na and P concentration in the plasma and decreased Ca and P concentrations in hens subjected to HS.

Acknowledgements: The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this research through Research Group Project No. RGP-267.

Author contribution: A.M. Abudabos, A.N. Al-Owaimer were in charge for planning and conducting the growth trial. E.O.S. Hussein, M.H. Ali and M.Q. Al-Ghadi were in charge of collecting samples and do the lab analysis. A.M. Abudabos was in charge of preparing the manuscript.

REFERENCES

Ajakaiye, J.J., A. Perez-Bello and A. Mollineda-Trujillo (2010). Impact of vitamins C and E dietary supplementation on leukocyte profile of layer hens exposed to high ambient temperature and humidity. Acta Vet. Brno. 79: 377–383.

Al-Saffar, A.A. and S.P. Rose (2002). Ambient temperature and the egg laying characteristics of the laying fowl. World's Poult Sci. J.58:317–331.

Balnave, D. and J. Brake (2005). Nutrition and management of heatstressed pullets and laying hens. World's Poult. Sci. J. 61:399–406.

Balogun, T.F., S,B, Afolayan and F.C. Njoku Dafwang II (1996). Effect of Ascorbic Acid and Acetylsalicylic Acid supplementation of broiler diets on performance during the hot season. Samaru J. Agri. Edu. 5:80–86.

Celik, L.B., A. Tekeli and O. Ozt¨urkcan (2004). Effect of supplemental L-carnitine in drinking water on performance and egg quality of laying hens exposed to a high ambient temperature. J. Anim. Physiol. Anim. Nut. 88:229–233.

Chand, N., S. Muhammad, R.U. Khan, I.A. Alhidary and Zia ur Rahman (2016). Ameliorative effect of synthetic γ-aminobutyric acid (GABA) on performance traits, antioxidant status and immune response in broiler exposed to cyclic heat stress. Environ. Sci. Poll. Res. 23:23930-23935.

Chand, N., S. Naz, H. Maris, R.U. Khan, S. Khan and M.S. Qureshi (2017). Effect of betaine supplementation on the performance and immune response of heat stressed broilers. Pakistan J. Zool. (in press) DOI: http://dx.doi.org/10.17582/journal.pjz/2017.49

Chand, N., S. Naz, A. Khan, S. Khan and R.U. Khan (2014). Performance traits and immune response of broiler chicks treated with zinc and ascorbic acid supplementation during cyclic heat stress. Inter. J. Biometeorol.58:2153-2157.

Daniel, M. and D. Balnave(1981).Response of laying hens to gradual and abrupt increases in ambient temperature and humidity. Aust. J. Exp. Agric. Anim. Husb. 21:189–195.

de Andrade A. N., Rogler JC, Featherston WR (1976). Influence of constant elevated temperature and diet on egg production and shell quality. Poult. Sci. 55:685–693

Dorr, P. and S.L. Balloun (1976) Effect of dietary vitamin A, ascorbic acid and their interaction on turkey bone mineralisation. Br. Poult. Sci. 17: 581–599

Khan, R.U., S. Naz and K. Dhama (2014a). Chromium: pharmacological applications in heat stressed poultry. Inter. J. Pharmacol. 10: 213-317.

Khan, R.U., Z.U. Rahman, I. Javed and F. Muhammad (2013).Supplementation of vitamins, probioitics and proteins on oxidative stress, enzymes and hormones in post-moulted male broiler breeder.ArchivTierzucht,61: 607-616.

Khan, R.U., S. Naz, Z. Nikousefat, M. Selvaggi, V. Laudadio and V. Tufarelli (2012).Effect of ascorbic acid in heat-stressed poultry. World's Poultry Sci. J. 68 (3): 477-490.

Khan, R.U., S. Naz, Z. Nikousefat, V. Tufarelli, M. Javadani, N. Rana and V. Laudadio (2011). Effect of vitamin E in heat-stressed poultry. World's Poultry Sci. J. 67(3):469-478.

Khan, R.U., Z.U. Rahman, I. Javed and F. Muhammad (2014b). Serum antioxidants and trace minerals as influenced by vitamins, protein and probiotics in male broiler breeders. J. Appl. Anim. Res. 42(3): 249-255.

Keshavarz K. (1996). The effect of different levels of vitamin C and cholecalsiferol with adequate or marginal levels of dietary calcium on performance and egg shell quality of laying hens. Poult Sci. 75:1227–1235.

Maini, S., S.K. Rastogi, J.P. Korde, A.K. Madan and S.K.Shukla (2007). Evaluation of oxidative stress and its amelioration through certain antioxidants in broilers during summer. J. Poultry Sci. 44: 339-347.

Mashaly, M.M., G.L. Hendricks, M.A. Kalama, A.E. Gehad, A.O. Abbas and P.H. Peterson (2004). Effect of heat stress on production parameters and immune responses of commercial laying hens. Poult. Sci. 83: 889-894.

Mignon-Grasteau, S., U. Moreri, A. Narcy, X. Rousseau,

T.B. Rodenburg, M. Tixier-Boichard and T. Zerjal (2105). Robustness to chronic heat stress in laying hens: a meta-analysis. Poultry Sci. 94:586–600.

Miller, P.C. and M.L. Sunde (1975).The effects of precise constant and cyclic environments on shell quality and other lay performance factors with leghorn pullets.Poult. Sci. 54:36-46.

Maurice DV, Lightsey SF, Abudabos AM, Toler JE. 2002. Factors affecting ascorbic acid biosynthesis in chickens: III. Effect of dietary fluoride on L-Gulonolactone oxidase activity and tissue Ascorbic Acid (AsA) Concentration. J Anim Phys Anim Nut 86: 383-388

Pardue, S.L. and J.P. Thaxton (1986). Ascorbic acid in poultry: A review. World's Poult. Sci. J. 42:107–123.

Puthpongsiriporn U, Scheideler SE, Sell JL, Beck MM (2001) Effects of vitamin E and C supplementation on performance, in vitro lymphocyte proliferation, and antioxidant status of laying hens during heat stress. Poult. Sci. 80:1190–1200

Ramnath, V., P.S. Rekha and Sujatha, K.S.(2008). Amelioration of heat stress induced disturbances of antioxidant defense system in chicken by Brahma Rasayana. Evidence- Based Compl. Alt. Med. 5 (Suppl. I): 77-84.

Raza T, Chand NR, Khan U, Shahid MS Abudabos AM (2016). Improving the Fatty Acid Profile in Egg Yolk Through The Use Of Hempseed (Cannabis Sativa), Ginger (Zingiber Officinale), and Turmeric (Curcuma Longa) In the Diet of Hy-Line White Leghorns. Arch. Anim. Breeding 59: 183-190.

SAS Institute Inc. 2009. SAS/STAT R 9.2. User's guide, 2nd ed. Cary, NC, SAS Institute Inc.

Seven, P.T. (2008).The effects of dietary Turkish propolis and vitamin C on performance, digestibility, egg production and egg quality inlaying hens under different environmental temperatures. Asian-Aust. J. Anim. Sci. 21:1164–1170.

Simon, M. S. (2003). Reducing heat stress problems. World Poultry, 19(3): 16-17.

Star, L., Decuypere, E., Parmentier, H.K. and Kemp, B. (2008). Effect of single or combined climatic and hygienic stress in four layer lines: 2. Endocrine and oxidative stress responses. Poultry Sci. 87: 1031–1038.

Tanor, M.A., S. Leeson and J.D. Summers (1984). Effect of Heat Stress and Diet Composition on Performance of White Leghorn Hens. Poultry Sci. 63:304-310

Torki, M.,S. Zangeneh and M. Habibian (2014). Performance, Egg Quality Traits, and Serum Metabolite Concentrations of Laying Hens Affected by Dietary Supplemental Chromium Picolinate and Vitamin C Under a Heat-Stress Condition. Biol. Trace Elem. Res. 157:120–129.

Waseem, A., A. Shakeel and Z. Kamran (2008).Response of layinghens to vitamin C supplementation through drinking waterunder sub-tropical conditions. Avian Biol. Res. 1:59–63.

Wolfenson, D., D. Bacharach, M. Maman, Y. Graber and I. Rozenboim. (2001). Evaporative cooling of ventral regions of the skin in heat-stressed laying hens. Poult. Sci. 80:958-964.